

REMARKS

In view of the comments which follow, reconsideration of the Official Action of October 21, 2003 is respectfully requested by Applicants.

A current Claims Listing dated 3/17/04 (2 pages) is submitted herewith.

Claim 44 has been amended to remove a hyphen between "receptor" and "bound".

Claims 44-52 are pending and stand rejected.

Rejection under 35 USC §102 (b)

In Paragraph 3 of the instant action, claims 44, 47-49, 51, and 52 have been rejected under 35 USC §102 (b) as being anticipated by Kuo, EP 813,064 A1 (hereinafter "Kuo"). The Examiner argues that Kuo discloses a solid support on which an antibody specific to an epitope of an analyte and a first labeled antibody, which is specific to another epitope of the analyte, are immobilized. A second labeled antibody is also provided which is specific to the first labeled antibody (abstract). The signal generated by the complex is detected on the substrate. According to one embodiment, there is a reagent region containing a second antibody labeled with gold sol, a second reagent region containing a third antibody labeled with gold sol, and a capture zone with immobilized first antibody (column 4). The support may also be provided with a positive control zone (column 5).

The present invention

The subject matter of the present invention is a method for multiepitope detection of an analyte in a sample, the analyte comprising two or more specific binding regions, i.e., epitopes. In the method for multiepitope detection according to the present invention, two or more test areas are provided that are spatially separated. On each of the test areas, exactly one receptor type is bound, the receptor type in each of the spatially separated test

areas being different from the receptor types of the other test areas. Each of the receptor types binds exactly to one specific epitope of the analyte. Thus, different epitopes of the analyte are addressed in the different test areas.

An important advantage of the inventive method is explained in the following. Having, e.g., an unspecific binding of an interfering substance to one of the receptors, the obtained signal in the corresponding test area can be identified as a false positive result due to unspecific binding since the receptors of the other test areas do not bind unspecifically to the interfering substance, and thus the other test areas do not produce a signal. If, on the contrary, multiple receptors are applied on one test area, as in the prior art, any unspecific binding of an interfering substance to any one of the receptors always results in a signal, and false positive results cannot be excluded.

Furthermore, it has been found that in test areas on which only one specific receptor type is present and thus only one epitope is bound, unspecific binding is considerably reduced. When different receptor types are each applied separately on individual areas, no measurable unspecific binding is observed, whereas a test area on which a mixture of several receptor types has been applied exhibits clearly measurable unspecific binding. The inventive combined evaluation of different spatially separated test areas which each contain one specific receptor type which binds specifically to one specific epitope of an analyte thus considerably improves the sensitivity and reliability of the inventive multiepitope detection method, especially by reducing false-positive results and by enabling the unequivocal recognition of truly positive results. The reliability of a test result is extremely important in the case of tests which have serious consequences for a patient and thus must meet high specificity requirements, e.g., an HIV test.

Additionally, the use of spatially distinct test areas each having bound one single receptor type advantageously enables the optimization of the test conditions for every test area, i.e., for each of the different receptor-epitope binding reactions. This provides a

further improvement of the sensitivity of the multiepitope detection according to the invention.

Kuo (EP 813,064 A1)

Kuo discloses a sandwich-type immunoassay wherein an analyte is trapped between two antibodies in a sandwich configuration. A fundamental difference of the sandwich-type immunoassay according to Kuo and the subject matter of the present invention is the fact that the Kuo test strip does not provide two or more spatially separated test areas. Furthermore, Kuo does not disclose spatially discrete test areas each having bound precisely one different type of receptor.

According to the method of Kuo, a first antibody specific for a first epitope of an analyte is bound (immobilized) to a solid support (column 1, lines 57-59). A primary signal generator bound to a second antibody which is specific to a second epitope of the analyte is *included in the test medium* (column 2, lines 1-4). Also *included in the test medium* is a secondary signal generator bound to an antibody specific for the second antibody (column 2, lines 6-8). The first antibody is immobilized in a discrete capture zone. The method of Kuo would not be operable if either the second or third antibody were bound, i.e., immobilized, to the test strip. The second and third antibodies must be mobile in order to flow along with the analyte to the detection zone, i.e., test area, to be bound by the first antibody (column 4, lines 12-16). The second and third antibodies are impregnated in the carrier – they are not bound to the carrier (column 5, lines 33-35).

Applicants argue that the Examiner's understanding of the number of antibodies immobilized on the solid support is in error (see italicized passage in previous paragraph). Kuo does not teach a solid support comprising a first and a second spatially separate test area, a first receptor bound to the first test area, and a second receptor bound to the second test area as recited in Applicants claims 44, 47-49, 51, and 52. Rather, Kuo teaches a single test area ("capture zone") and a single receptor bound ("immobilized") to

that test area. The paragraph bridging columns 4 and 5, as well as Figure 2, teach a nitrocellulose test strip (10) having a wicking pad (1), a reagent region (3) containing Ab2, a second reagent region (5) containing Ab3, and a capture zone (7) in which there is immobilized Ab1. Test fluid is applied to the wicking pad where it is absorbed and begins its flow up the strip through zones 3 and 5 and eventually to detection zone 7 where the detectable signal from the signal generator is observed. Ab2 and Ab3 cannot be bound or immobilized to the reagent regions, otherwise the test described by Kuo would not be operable. Kuo describes an optional embodiment having a positive control zone (9) containing an immobilized specific binding partner for either labeled Ab2 or Ab3. This binding partner, however, does not bind specifically with the analyte as required in Applicants' claimed invention. Kuo teaches that only one analyte-specific antibody is bound to the solid phase. A second, analyte-specific antibody bound to a separate test area is not taught or suggested by Kuo.

In paragraph 11 of the instant action, the Examiner argues that Applicants' arguments are unconvincing. The Examiner states that the reference "clearly teaches multiple spatially separate regions with different immobilized receptors in each". As explained above, Applicants' respectfully disagree and, if necessary, invite the Examiner to point out the specific passage or passages in Kuo where such is taught.

Since Kuo does not teach a solid support comprising a first and a second spatially separate test area nor a first receptor specific for an analyte and bound to a first test area and a second receptor specific for the analyte and bound to a second test area, Kuo cannot anticipate Applicants' invention. Applicants respectfully request the Examiner's reconsideration of his rejection of claims 44, 47-49, 51, and 52.

In Paragraph 4 of the instant action, claims 44, 45, 49, and 51 have been rejected under 35 USC §102 (b) as being anticipated by Bellet et al., U.S. Patent No. 5,011,771 (hereinafter "Bellet"). The Examiner argues that Bellet discloses an immunometric assay comprising the formation of a complex between antigen and multiple immobilized

monoclonal antibodies against different epitopes of the antigen and with a detectably labeled monoclonal antibody. According to the reference, the Examiner argues, it is important that the multiple immobilized antibodies be bound in close proximity (column 8, lines 7-9).

Bellet (US 5,011,771)

Bellet teaches a multiepitopic immunometric assay in which a single test area is provided having bound thereto a mixture of at least two different antibodies on an insoluble solid phase and using one of said two antibodies in a soluble, detectably labeled form. A sandwich assay is then carried out with this configuration (column 3, lines 33-37). Bellet teaches away from the use of only one immobilized monoclonal antibody (column 3, lines 38-44). Bellet does not teach or even suggest a solid phase comprising a non-porous support and first and second spatially separate test areas, nor does Bellet teach or suggest a first receptor specific for an analyte bound to a first test area and a second receptor specific for the analyte bound to a second test area.

In the Examiner's response to Applicants' previous arguments, the Examiner notes that the disclosure of multiple immobilized antibodies disposed in close proximity is interpreted as meeting the limitation of spatially separate test areas. Applicants' respectfully disagree and argue that this interpretation is in error. Applicants point out that the skilled artisan would not make such an interpretation. The skilled artisan would interpret Bellet's solid phase having bound thereto a mixture of at least two different antibodies as defining a single test area, i.e., one area in which a reaction takes place and is confined. Support for Applicants' position is the hypothetical explanation Bellet sets forth in Figure 1 to explain the results observed. That is, upon binding of the antigen to the solid phase via both antibodies 1 and 2, additional epitopes are exposed due to a conformational change, thereby permitting binding of the antigen to the labeled antibody (column 4, lines 40-47). Further, Bellet teaches the synergistic combination of multiple different antibodies on the solid phase showing substantial binding whereas any one of

them does not (column 3, lines 41-44). Even further, Bellet teaches that close proximity of the multiple immobilized antibodies is important (column 8, lines 7-11). The skilled artisan would interpret this as teaching a single test area comprising multiple immobilized antibodies, especially in light of the specific examples (column 11, lines 61-63).

In their specification, Applicants' further describe the spatially separate test areas of their invention, the distance between them being selected such that the applied reagents cannot coalesce. A distance between the edges of test areas is taught to be 0.05 to 5 mm. There is an inert surface between the areas which can neither bind to the analyte nor to other sample components (page 10, lines 1-5). Even apart from the clear description as taught by Applicants, one skilled in the art to which the present invention belongs would interpret "spatially separate" to mean "separated by space".

Since Bellet does not teach a solid support comprising a first and a second spatially separate test area nor a first receptor bound to the first test area and a second receptor bound to the second test area, there being no more than one analyte-specific receptor bound per test area, Bellet cannot anticipate Applicants' invention. Applicants respectfully request the Examiner's reconsideration of his rejection of claims 44, 45, 49, and 51.

Rejections under 35 USC §103 (a)

In Paragraph 8 of the instant action, claims 45, 46, and 50 have been rejected under 35 USC §103 (a) as being unpatentable over Kuo, EP 813,064 A1 (hereinafter "Kuo"). The Examiner argues that the reference teaches an immunoassay method as previously discussed under 35 USC §102 (b). However, the reference does not teach specific analytes or the size of the test area. The Examiner argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to use test areas with diameters less than 1 mm and assay for specific analytes with the method and kit of Kuo.

In rebuttal, Applicants argue that the Examiner's case for *prima facie* obviousness has not been made. Claims 45, 46, and 50 depend from independent claims 44 and 49, respectively. Patentability of claims 44 and 49 has been argued under 35 USC §102 above, and dependent claims 45, 46, and 50 should enjoy the same patentability as the claims from which they depend. Applicants respectfully request the Examiner's reconsideration of this ground for rejection of claims 45, 46, and 50.

In paragraph 9 of the instant action, claims 46-48, 50, and 52 have been rejected under 35 USC §103 (a) as being unpatentable over Bellet et al., U.S. Patent No. 5,011,771 (hereinafter "Bellet"). The Examiner argues that the reference teaches a multipitopic assay as previously discussed under 35 USC §102 (b). However, the reference does not teach the diameter of the test area, a control area, or latex particles as the label. The Examiner argues that it would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to use a control area and latex particles as the label with the method and kit of Bellet.

In rebuttal, Applicants argue that the Examiner's case for *prima facie* obviousness has not been made. Claims 46-48, 50, and 52 depend from independent claims 44, 49, and 51, respectively. Patentability of claims 44, 49, and 51 has been argued under 35 USC §102 above, and dependent claims 46-48, 50, and 52 should enjoy the same patentability as the claims from which they depend. Applicants respectfully request the Examiner's reconsideration of this ground for rejection of claims 46-48, 50, and 52.

Applicants submit that their application is now in condition for allowance, and favorable reconsideration of their application in light of the above amendments and remarks is respectfully requested. Allowance of claims 44-52 at an early date is earnestly solicited.

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The Examiner is hereby authorized to charge any fees associated with this
Amendment to Deposit Account No. 50-0877. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

A handwritten signature in cursive script, reading "Marilyn L. Amick", written over a horizontal line.

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CLAIMS LISTING 3/17/2004

- 1-43. (previously cancelled)
44. (currently amended) A method for multiepitope detection of an analyte in a sample, the analyte comprising at least two epitopes, comprising the steps of:
- (a) providing a solid phase comprising a non-porous support, a first and a second spatially separate test area, and a first and a second receptor, the first and second receptors binding specifically with said analyte but to different epitopes of the analyte, the first ~~receptor bound~~ receptor bound directly or indirectly to the first test area and the second receptor bound directly or indirectly to the second test area, there being no more than one analyte-specific receptor bound per test area,
 - (b) contacting the sample with the solid phase and with a detection reagent comprising a third receptor that binds with the analyte and that is bound to a signal generating group, and
 - (c) determining presence or amount of the signal generating group bound to the test areas via the analyte as a measure of the analyte in said sample.
45. (previously amended) The method of claim 44 wherein the analyte is selected from the group consisting of HIV I, HIV II, HBV, and HCV antibodies and HIV antigens.
46. (original) The method of claim 44 wherein each test area has a diameter of 0.01 to 1 mm.
47. (original) The method of claim 44 wherein the solid phase further comprises a control area.

CLAIMS LISTING 3/17/2004

48. (original) The method of claim 44 wherein said detection reagent is a universal detection reagent comprising labelled latex particles.
49. (previously amended) A solid phase for multiepitope detection of an analyte in a sample, the analyte comprising at least two epitopes, the solid phase comprising a non-porous support, a first and a second spatially separate test area, and a first and a second receptor, the receptors binding specifically to the analyte but to different epitopes of the analyte, the first receptor-bound directly or indirectly to the first test area and the second receptor bound directly or indirectly to the second test area, there being no more than one analyte-specific receptor bound per test area.
50. (original) The solid phase of claim 49 wherein each test area has a diameter of 0.01 to 1 mm.
51. (previously amended) A test kit for multiepitope detection of an analyte in a sample, the analyte comprising at least two epitopes, the test kit comprising a solid phase of according to claim 49 and a detection reagent comprising a third receptor that binds with the analyte and that is bound to a signal generating group.
52. (original) The test kit of claim 51 wherein said detection reagent is a universal detection reagent comprising labelled latex particles.
- 53-69. (previously cancelled)